

Hydrogen Enzyme Electrode for Bio-Fuel Cell Applications.

A.A. Karyakin¹, S.V. Morozov¹, E.E. Karyakina¹,
S. Cosnier², N.A. Zorin³

- ¹ Faculty of Chemistry, M.V. Lomonosov Moscow State University, 119899, Moscow, Russia
- ² LEOPR, UMR CNRS 5630, Université Joseph Fourier BP 53, 38041 Grenoble Cedex 9, France
- ³ IBBP RAN, 142190, Moscow region, Puschino, Russia

The enzymes responsible in nature for oxidation/evolution of molecular hydrogen are trivially called hydrogenases. The phenomenon of direct bioelectrocatalysis by hydrogenases has been shown in the beginning of 80s [1]. In particular, hydrogenase of *Th. roseopersicina* being adsorbed on carbon black electrodes catalyzed electrochemical oxidation/evolution of hydrogen in the absence of any diffusion-free immobilized mediators. However, in early investigations the electrode supports used were not mechanically stable and, thus, the resulting hydrogen enzyme electrodes could not be considered for their potential applications in biofuel cells. The present investigation has been carried out using the carbon filament material (CFM) specially designed by Russian State Company ‘Alten’ for development of fuel cells.

A procedure of hydrogen enzyme electrode preparation includes the pretreatment of electrode support followed by enzyme immobilization. Electrode pretreatment is a crucial step, especially taking into account, that initially CFM is completely hydrophobic. The optimal hydrophilization procedure was the treatment with sulfuric acid for 10-30 min followed with buffer washing for 48 h.

After immersion of the enzyme electrode in neutral buffer solution saturated with molecular hydrogen, the equilibrium hydrogen potential has been achieved in less than 5 minutes. In contrast to blank carbon electrode (fig. 1) the hydrogen enzyme electrode was characterized by high values of positive current in positive potential region. In the absence of molecular hydrogen, however, the only cathodic current was observed in the entire potential range investigated (fig. 1). Thus, the anodic current is due to hydrogen oxidation on hydrogenase electrode, and the standard potential observed is a hydrogen equilibrium potential. This confirms our previous results achieved on carbon black electrode concerning the equilibrium hydrogen potential on hydrogenase electrode, which, unfortunately, has not been reported by other laboratories. Positive wave of the current-potential curve is not linearized in the traditional Tafel plots. It seemed that both electrochemical stages of electron transfer between the enzyme active sites and the electrode were the limiting ones. Indeed, the steady-state current-voltage curves were fit to the two-exponential equation evaluated for the two sequential one-electron electrochemical stages.

$$i = \frac{\exp\left(2\frac{\alpha F}{RT}\eta\right) - \exp\left(-2\frac{(1-\alpha)F}{RT}\eta\right)}{K_1 \exp\left(\frac{\alpha F}{RT}\eta\right) + K_2 \exp\left(-\frac{(1-\alpha)F}{RT}\eta\right)}$$

,where K₁ and K₂ are come constants including the surface concentration of active enzyme and definite combinations of elementary constants. We let α=0.5, as for most electrochemical reactions. The optimization of the polarization curve positive branch in accordance with equation by two parameters K₁ and K₂ resulted in a good fit with correlation coefficient exceeded 0.999.

The important characteristic of electrocatalyst is the exchange current of the corresponding catalyzed reaction. This value can be found as from Tafel plots, as from the K₁ and K₂ of the above equation. Table 1 summarizes the

values of the exchange current calculated both per electrode unit and per molecule of the catalyst. It is seen, that in neutral aqueous solutions platinum electrode possesses similar electrocatalytic activity relatively the electrode area. However, when recalculated the exchange current per molecule of the catalyst, the effectivity of the enzyme becomes two order of magnitude higher.

A crucial factor for biotechnology applications is the stability of the enzyme electrode. Hydrogenase immobilization onto the CFM causes the dramatic improvement of both operational and storage stability. It is seen in fig. 2, that even after half a year of storage with periodical testing, the enzyme electrode preserved more than 50% of its initial activity.

We conclude, that hydrogen enzyme electrode based on hydrogenase immobilized on CFM is applicable for biofuel cell development. The enzyme electrode is characterized by high effectivity: hydrogen equilibrium potential and high current of hydrogen oxidation. The exchange current per molecule of the catalyst is two order of magnitude higher as compared to the ‘best’ known electrocatalyst platinum.

Table 1.

	Exchange current, μA/cm ²	Exchange current per molecule of the catalyst, A·10 ⁺¹⁹
Hydrogenase electrode	11±1	8±1
Pt (pH 7.0)	<10	<0.1

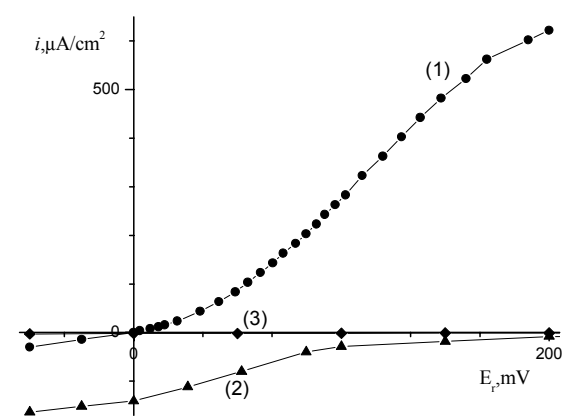


Fig. 1. Hydrogenase CFM electrode in H₂ (1), Ar (2) and CFM blank electrode (3).

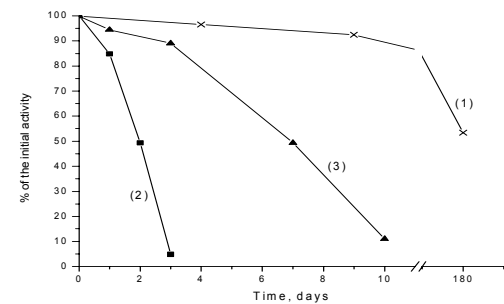


Fig. 2. Stability: (1) hydrogenase CFM electrode, (2, 3) hydrogenase in solution, (3) with stabilizers.

[1]A.I.Yaropolov, A.A.Karyakin, S.D.Varfolomeyev, I.V. Berezin. *Bioelectrochem. Bioenerg.* **12** (1984) 267-77.
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